Reactive oxygen species in plant cells: their formation, localizatıon and functions

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The formation of reactive oxygen species, their localization in the cell, function and the role in the signal transduction have been discussed in the article.

Keywords: Stress, reactive oxygen species, signal transduction, singlet oxygen, hydrogen peroxide

One of the plant responses to adverse environmental conditions is the formation of reactive oxygen species (ROS) (Apel, Hirt, 2004; Foyer, Noctor, 2005, Sharma et al., 2012). ROS are considered to be intermediate products of normal cellular metabolism. The balance between the formation and elimination of ROS is disrupted during stress. Reactive oxygen species are formed during enzymatic oxidation of substances, as a result of the light-induced reactions due to one-, two-, three-electron oxygen reduction (Blokhina et al., 2003; Miller et al, 2010). Excessive accumulation of ROS in the cell depending on the stress intensity and duration inactivates enzymes, damages the plant vital organs, destroys the membranes, leads to the degradation of pigments, proteins, lipids, and nucleic acids, which ultimately results in cell death. Besides, ROS act as messengers in the signal transduction. Reactive oxygen species are divided into 2 groups: free radicals - superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), peroxide radical ($RO_2^{\bullet}$) and neutral molecules - hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), ozone (O_3), etc. (Asada, 1999; Noctor et al., 2015).

Superoxide anion radical ($O_2^{\bullet-}$): The primary reduction product of molecular oxygen-superoxide anion radical ($O_2^{\bullet-}$) is formed mainly in electron transport chain (ETC) of chloroplasts and mitochondria as well as in peroxisomes, plasmalemma, and apoplast (Sharma et al., 2012; Demidchik, 2015). The formation of $O_2^{\cdot-}$ in chloroplasts occurs due to the function of the I and II photosystems. So in PSI, it occurs at the expense of 4Fe-4S-cluster, ferredoxin (Fd), ferredoxin-NADPH-reductase and in PSII at the expense of P680, pheophytin, and plastoquinone (Apel, Hirt, 2004; Raja et al., 2017). In mitochondria, $O_2^{\bullet-}$ is formed in the I and III respiration chains (Miller et al., 2001), in peroxisomes due to the oxidation of xanthine by xanthine oxidase (Miller et al., 2010). The generation of superoxide radical in the endoplasmic reticulum (ER) related to the oxidation of cytochrome P-40 and NADPH in the presence of cytochrome c.

In plasmalemma, $O_2^{\cdot-}$ is formed at the expense of the NADPH oxidation (Mittler, 2002; Scandalios, 2002). Some authors indicate the formation of superoxide radical in apoplast due to the action of peroxidases, glucose oxidases, amino acid oxidases and autoxidation of phenols, quinones, flavines and glutathione (Lukatkin, 2002; Demidchik, 2015; Sharova, Medvedyev, 2017). The lifespan of superoxide radical is very short (approximately 1 microsecond). It almost cannot penetrate through the membrane and has no strong oxidative properties (Sharma et al., 2012). Superoxide radical is considered to be a source of the more toxic, reactive species of oxygen, such as $HO_2^{\bullet}$ and H_2O_2 (Gill, Tuteja, 2010).

Hydroperoxide radical ($HO_2^{\bullet}$): Hydroperoxide radical is a protonated form of superoxide anion radical. It is formed in the acidic medium due to the interaction with H_2O_2, which leads to the protonation of $O_2^{\bullet-}$ or as a result of water radiolysis (Gill, Tuteja, 2010). Being a strong oxidizing compound it has the ability to oxidize organic molecules by penetrating the cell.

Hydrogen peroxide (H_2O_2): Having a moderate oxidation capacity, this compound is considered to be a relatively stable form of ROS (Lukatin, 2002). It is formed when 2 electrons bind molecular oxygen or by the dismutation of two $O_2^{\bullet-}$ molecules. H_2O_2 is formed mainly in chloroplasts, mitochondria, peroxisomes, endoplasmic reticulum and plasmalemma (Miller et al., 2010). It is formed in chloroplasts by water oxidation (Kreslavski et al., 2012), due to oxidation of glycolate by glycolate oxidase or flavin oxidases in peroxisomes, and β-oxidation of fatty acids in the presence of acetyl-CoA-oxidase in glyoxysomes (Reczek, Chandel, 2015; Raja et al., 2017). The formation of hydrogen peroxide in mitochondria occurs due to the action of mitochondrial superoxide dismutase (Sharma et al., 2012), whereas in apoplast this compound is formed with the involvement of diamine oxidases and

polyamino oxidases (Garifzyanov et al., 2011). This compound is produced also by oxalate oxidases, peroxidases, and NADPH-oxidases (Apel, Hirt, 2004).

The reactivity of H_2O_2 is considered to be moderate. H_2O_2 can pass through the membranes and thereby act as a messenger in the stress signaling response (Halliwell, 2006; Moller et al., 2007). In plants, ROS are formed in various metabolic pathways and they can also be produced under stress conditions in various cellular compartments, including chloroplasts, mitochondria, peroxisomes, the endoplasmic reticulum (ER), and plasma membranes (Table 1).

***Hydroxyl radical (OH•)**:* This compound is a product of three-electron oxidation of oxygen, has a short lifespan (10^{-9} s), a strong oxidation potential, and can interact with all biological macromolecules (Gill, Tuteja, 2010; Demidchik, 2015).

Its formation in the cell is mainly due to the Haber-Weiss (interaction between H_2O_2 and $O_2^{•-}$ and Fenton reactions (during oxidation of metals with variable valencies). Generation of OH• radicals occurs due to the interaction of hydrogen peroxide, ferredoxin and ubiquitin and as well as water radiolysis (Sharova, Medvedyev, 2017).

***Singlet oxygen ($^1O_2$)**:* Highly reactive singlet oxygen is formed in the chloroplasts due to the reactions with the involvement of chlorophyll and flavins (Foyer and Noctor, 2005). This compound can also be formed as a result of the dismutation of ROS, such as $O_2^{•-}$, OH•, $HO_2^•$, Haber-Weiss reactions, and the action of the enzymes superoxide dismutase, catalase and peroxidase. Oxygen is one of the more reactive forms of ROS, with a very short lifespan (about 3 microseconds), and the ability to diffuse to other parts of the cell (Gill, Tuteja, 2010).
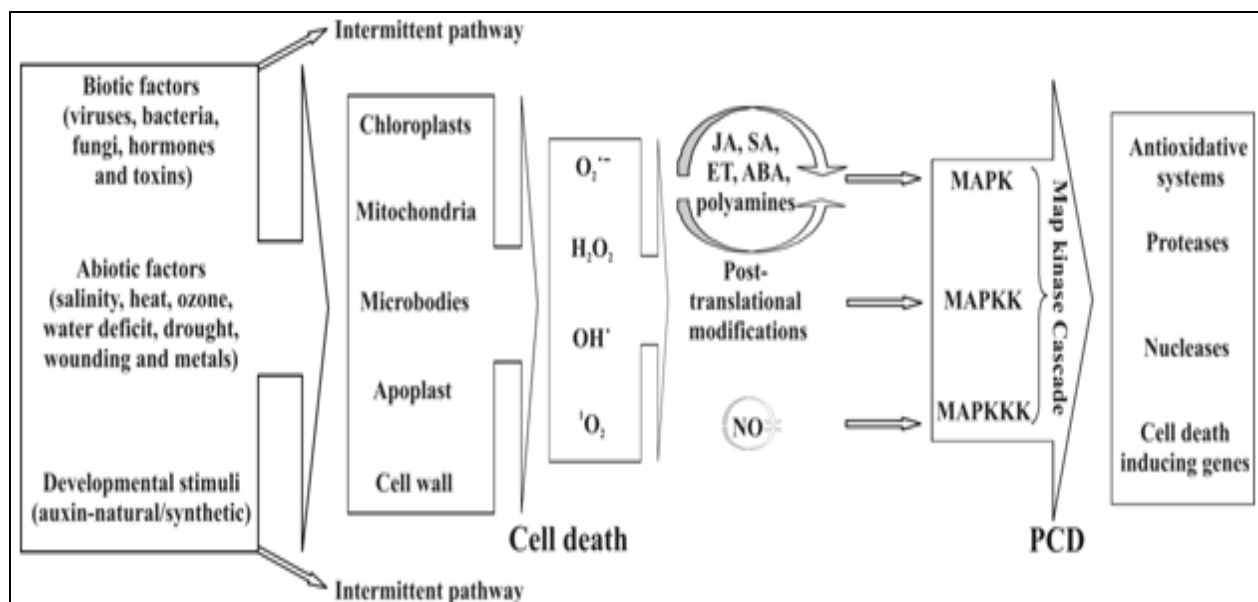
Table 1. Key reactive oxygen species (ROS), their properties, and main scavenging systems in plant cells

ROS	Half-life and mobility	Mode of action	Cellular sources	Main scavenging systems
Superoxide radical ($O_2^{•-}$)	1 μs, 30 nm	Reacts with double bond containing compounds such as iron-sulphur (Fe-S) clusters of proteins; reacts with nitric oxide (NO) to form peroxynitrite ($ONOO^-$)	Formed in many photooxidation reactions (flavoprotein, redox cycling), Mehler reaction in chloroplasts, mitochondrial electron transport chains (ETCs) reactions, glyoxisomal photorespiration, peroxisomes, and plasma membrane. NADPH oxidase in membranes. Xanthine oxidase and membrane polypeptides in peroxisomes. Reactions of ozone (O_3) and $OH^•$ in apoplastic space	Superoxide dismutases (SODs)
Hydroxyl radical ($OH^•$)	1 ns, 1 nm	Extremely reactive with protein, lipids, DNA, and other macromolecules	Reaction of H_2O_2 with $O_2^{•-}$ (Haber-Weiss reaction), reactions of H_2O_2 with Fe^{2+} (Fenton reaction). Decomposition of O_3 in apoplastic space	Flavonoids, prevention of $OH^•$ formation by sequencing Fe
Hydrogen peroxide (H_2O_2)	1 ms, 1μm	Oxidizes proteins; reacts with $O_2^{•-}$ in a Fecatalyzed reaction to form $OH^•$	ETCs of mitochondria, chloroplasts, endoplasmic reticulum, and plasma membrane. Photorespiration, fatty acid β-oxidation, urate oxidase, and MnSOD in peroxisomes	Catalases, various Peroxidases and flavonoids
Singlet oxygen ($^1O_2$)	1 μs, 30 nm	Directly oxidizes protein, polyunsaturated fatty acids, and DNA	Photoinhibition, photosystem II electron transfer reactions in chloroplasts	Carotenoids and α-tocopherols

The role of ROS in the plant cells: ROS are involved in the growth and development of plants, in photosynthesis, respiration, programmed cell death, plant responses to stress factors and signal transduction processes (Guan et al., 2000; Zhou et al., 2012; Demidchik et al., 2018). As free radicals and singlet oxygen have short lifespans and are unable to migrate to other parts of the cell, their functions in the cell have not been studied sufficiently at present. Some authors described the role of $O_2^{\bullet-}$ and $OH\bullet$ radicals in the growth processes and the plant defense against pathogens (Künstler et al., 2015; Sharova, Medvedyev, 2017). $^1O_2$ is involved in the induction of the aging process (Passardi et al., 2004), $OH\bullet$ in the softening of cell walls during fruit ripening (Fry et al., 2001). Hydrogen peroxide, which differs from other active forms of oxygen in a relatively long lifespan and low reactivity, can penetrate through the cell wall and plays an important role in signal transduction. Thus, this compound performs a function of the second messenger in the NADPH oxidase signal system by the regulation of Ca channels and activates one of the kinase isoforms in the MAP-kinase signaling cascade (Demidchik et al.. 2015; Demidchik et al., 2018). H_2O_2 is involved in the regulation of growth and differentiation (Sharova, Medvedyev, 2017), seed germination (Gomes, Garcia, 2013), and leaf aging (Polesskaya, 2007) processes. H_2O_2 participates also in the regulation of the antioxidant enzyme activities and expression of their genes (Slesak et al., Künstler et al.,

2015), in the synthesis of the heat and cold shock proteins (Bhattacharjee, 2005), in plant responses to pathogen effects and lignification processes. Its rapid and excessive accumulation in areas affected by the pathogen initiates the processes leading to programmed cell death, resulting in necrotic spots that limit the spread of the infection in the plant (Lehmann et al., 2015). Moreover, H_2O_2 enhances the expression of genes of pathogen-induced (PR) proteins, resulting in Induced Systemic Resistance of the plant (Torres, 2010). The disrupted balance between the formation and utilization of ROS leads to excessive accumulation of ROS in the cell and lipid peroxidation. The main targets of ROS in the plant cell are membrane lipids and unsaturated fatty acids (Gill, Tuteja, 2010). Activating nucleosomes, ROS damage the carbohydrate bridges between nucleotides, which can lead to the destruction of DNA and RNA chains (Reczek, Chandel. 2015; Mittler, 2017). ROS disrupt the membrane structure, reduce the activity of enzymes in the chloroplast, weaken the efficiency of the electron transport chain by damaging FS1 and FS2 (Gill, Tuteja, 2010).

The role of ROS in signal transduction: For affecting plant growth and cell metabolism, ROS use other signaling pathways or molecules. According to some authors, plant hormones are positioned downstream of the ROS signal. Thus, H_2O_2 is known to induce the accumulation of plant stress hormones, such as JAs, SA, and ET (Fig. 1).

Fig. 1. Schematic representation of reactive oxygen species (ROS)-dependent cell death pathways in plants. Abbreviations: $^1O_2$: singlet oxygen; H_2O_2: hydrogen peroxide; MAPKK: mitogen-activated protein kinase kinase; MAPKKK: mitogen-activated protein kinase kinase kinase; $O_2^{\bullet-}$: superoxide radical; $OH^{\bullet}$: hydroxyl radical. (Karuppanapandian et al., 2011)

Besides the downstream localization of plant hormones of the ROS signal, they are also secondary messengers in many hormone signaling pathways (Pei et al., 2000; Orozco-Cardenas et al., 2001). ROS are central components in the plant stress responses. Depending on their concentrations, ROS have dual functions: ROS can induce defense genes and adaptive responses at low concentrations, whereas, at high concentrations, they initiate cell death (Neill et al., 2002). Sub-lethal concentrations of ROS were found to acclimate plants to biotic and abiotic stress conditions and reduce plant growth, which is considered as a part of an acclimatization mechanism (Neill et al., 2002; Torres et al., 2002; Benderradji et al., 2011). The recent reports on the role of ROS as signal molecules in growth and morphogenesis suggest that ROS are not only stress signal molecules but also an intrinsic signal in plant growth and development. MAPKs are also important transducers of the stress signal. They act upstream of the oxidative burst during O_3-treatment and the HR. But MAPKs can also be involved in ROS-dependent cell death. A MAPKKK of alfalfa (*Medicago sativa*) was found to activate cell death induced by H_2O_2 through a specific MAPK-scaffolding action (Nakagami et al., 2004). Although in the transgenic lines, functional plant homologs protect against ROS-mediated programmed cell death (Chen and Dickman, 2004). An evolutionarily conserved Arabidopsis BCL2-associated athano protein is induced by H_2O_2 and is capable to provoke PCD in yeast (*Saccharomyces cerevisiae*) and plants (Kang et al., 2006).

Thus, ROS are formed in the cell under normal conditions and participate in metabolism. Violation of pro- and antioxidant imbalances caused by unfavorable environmental factors leads to excessive accumulation of ROS in the cell and peroxidation of lipids, which leads to macromolecular damage and cell death. The enhanced content of ROS in the cell trigger also the defense mechanisms, the most important of which is the antioxidant defense system (Noctor et al., 2015).

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Bitki hüceyrələrində oksigenin fəal formaları: əmələ gəlməsi, lokalizasiyası, funksiyaları

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Oksigenin fəal formalarının əmələ gəlməsi, hüceyrədə lokalizasiyası, funksiyası və eyni zamanda hüceyrədə siqnalların ötürülməsində OFF-nin rolu icmal məqalədə şərh olunur.

Açar sözlər: *Stress, okssigenin fəal formaları, siqnalların ötürülməsi, sinqlet oksigen, hidrogen peroksid*